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Histological Alterations in the Liver of *Heteropneustes fossilis* (Bloch, 1794) Exposed to Polystyrene Nanoparticles

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Plastic pollution has been a serious environmental threat through its increasing application in every field whether it is household or industry. Plenty of plastic in aquatic debris ends up in aquatic environments where it degrades and fragments are nanosized through various ways. These nanoparticles can travel to almost all locations within the organisms via endogenous transport to cause severe damage to them. Among nanoplastics, polystyrene is creating more health hazards in aquatic organisms. Fishes are the best indicator of aquatic pollution as they get exposed to these toxicants by the unscrupulous use of plastic due to its easy applications in personal and public life. In the present study, *Heteropneustes fossilis* was exposed to various concentrations of polystyrene nanoparticles for 20 days to investigate the histological alterations in the liver under a Scanning Electron Microscope.

Keywords: Polystyrene nanoparticles; histology; Heteropneustes fossilis; scanning electron microscope.

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1. INTRODUCTION

Plastic production and its uses have increased tremendously in recent years (Guimaraes et al. 2000) [1]. Plastics are made up of a wide range of polymers such as polystyrene, polypropylene, polyethylene terephthalate and polyvinyl chloride [2,3]. One of the most widely used plastics is polystyrene and it is commonly called thermocol. Polystyrene is commonly used for moulding or expanding into foams as cosmetics, disposable plates, cups, trays, toys, food containers, medical equipment, etc. [4,5,6,7]. In inefficient waste management and recycling process, huge amounts of plastic debris enter the environment [8] by the process of degradation into large microplastic-5mm to 1mm in diameter, small microplastic -1mm to 1um and nanoplastic ≤ 1um in diameter [9]. Nanoplastics are emerging contaminants in the aquatic environment. Among Nanoplastics polystyrene creating more havoc and health hazard to aquatic animals. Polystyrene is an aromatic polymer formed as a result of polymerization of styrene monomers [7]. Polystyrene is non biodegradable, for that reason its products are great source of environmental pollution [10,11,12,7].

The accumulation of nanoplastics in the aquatic system is undergoing a continuous process of contamination which causes many harmful effects on aquatic organisms, especially on fish. Fish can ingest nanoplastics directly or via praying on other organisms or eventually enter through the food chain [13,14,15,16] and greatly affects vital organs such as the liver, intestine, and pancreas [17,18].

Heteropneustes fossilis (H. fossilis) is selected for the present study due to its easy availability and adaptability in laboratory conditions. H. fossilis is good indicator of environmental contaminants and gets adversely affected by environmental toxicants

Many researchers have studied several aspects of the toxicity of PS-NPs on the various tissues of the fishes but studies on the effect of 100nm size PS-NPs toxicity on the liver of H.fossilis is meagre. Therefore this study has been undertaken to investigate the hepatotoxicity of this toxicant.

2. MATERIALS AND METHODS

Materials: Healthy and adult catfish H. fossilis were selected for experiment and were collected from local fish market Dumka. The fishes selected for experiment were 10-15 cm in length and average weight 30-35gm and were maintained in large size fish tank of 84.4cm×53.35cm×53.35cm diamension. Polystyrene Nanoparticles size 100nm (manufactured in Sigma-Aldrich, Buchs, Switzerland), Paraformaldehyde (96%), Gluteraldehyde (25%), di-Sodium Hydrogen orthophosphate, Sodium dihydrogen orthophosphate (dihydrate) were purchased from Angel Scientific Stores.

Experimental design: The fishes selected for experiment were acclimatized for 15 days before experimentation in a large tank in the laboratory. During acclimatization, fishes were continuously aerated and maintained natural photoperiod. The fish were fed with commercial nutritious food once a day. The tank was cleaned thoroughly and filled with fresh water. After 15days of acclimatization, healthy fishes were taken out and divided into five groups: One Control and four PS-NPs treated and transferred in five different fish tank. Each fish tank 58.35 cm×39.46 cm ×39.46 cm diamension with 50 L dechlorinated tap water contained ten fishes. One group was kept for control and other four for experimental setup. Experimental fishes were exposed to a freshly prepared stock solution of Polystyrene nanoparticles with different dosages as 10µg/L, 20µg/L, 30µg/L, and 40µg/L. Control without test toxicant PS-NPs were run simultaneously. During the exposure period of 20 days, the control and PS-NPs treated tank were subjected to the same environmental conditions. During experimentation, the experimental tanks were renewed completely with a fresh solution of the same concentration in two-day intervals. Aerators were used to maintain the levels of dissolved oxygen (DO) in both the control and experimental groups. After completion of the exposure period, fish were sacrificed for the histological study of the liver. All experimental processes were in accordance with NIH Guide for the Care and Use of Laboratory animals.

Sampling: After 20 days of exposure, three fishes selected from each fish tank (control and various doses of PS-NPs) were collected and anaesthetized with clove oil [19,20] mixed in cold water, and their liver was dissected out and fixed in respective fixative accordingly and finally proceeded for histological observation on Scanning Electron microscope (SEM).

Histological analysis: For SEM study liver samples were fixed in Karnovsky's Fixative (mixture of 2% paraformaldehyde and 2.5% glutaraldehyde prepared in 0.1M phosphate buffer, pH 7.4) for 2-6 hours at 4°C and after washing with 0.1M phosphate buffer, it was post-fixed with 1% osmium tetraoxide solution (prepared in phosphate buffer, 0.2M and pH 7.4) for 2h at 4°C. After fixation samples were dehydrated through a graded series of acetone. Then tissues were dried using liquid CO_2 at a critical point drier at 31.5° C at 1100 psi, mounted on aluminium stubs followed by sputter coating using gold to coat samples with 20-30 nm thick film for observation under SEM (EU 018) at the Electron Microscope Facility Centre, SAIF, AIIMS, New Delhi, India.

3. RESULTS

The liver of the control fish showed a normal palisade arrangement of hepatocytes under SEM observation. Hepatic cords and mucus mass over hepatocytes clearly visible under SEM Study. Columns of hepatocytes extend from the portal region to central veins with well-demarcated sinusoids. Endothelial cells were flattened in shape. The round nuclei were seen. Leucocytes and kupffer cells frequently adhere to endothelium. Hepatic cords were seen as net-like structures.

Sinusoidal openings were small in size. The Bile canaliculi run along the entire cell surface. Portal space was very distinctly observed under SEM. In the control group, no pathological alterations were observed under SEM (Fig. 1).

The normal architecture of liver organization showed abnormal features when treated with test toxicant Polystyrene Nanoparticles. The structure of hepatic lobules had been changed.

in comparison with the control group (Fig. 2) under SEM observation. Hepatic lobules are identified as irregular polyhedral structures. Endothelium became irregular in shape. In low concentrations of PS-NPs (10µg/L), kupffer cells increase in number. Sinusoidal openings became large in shape

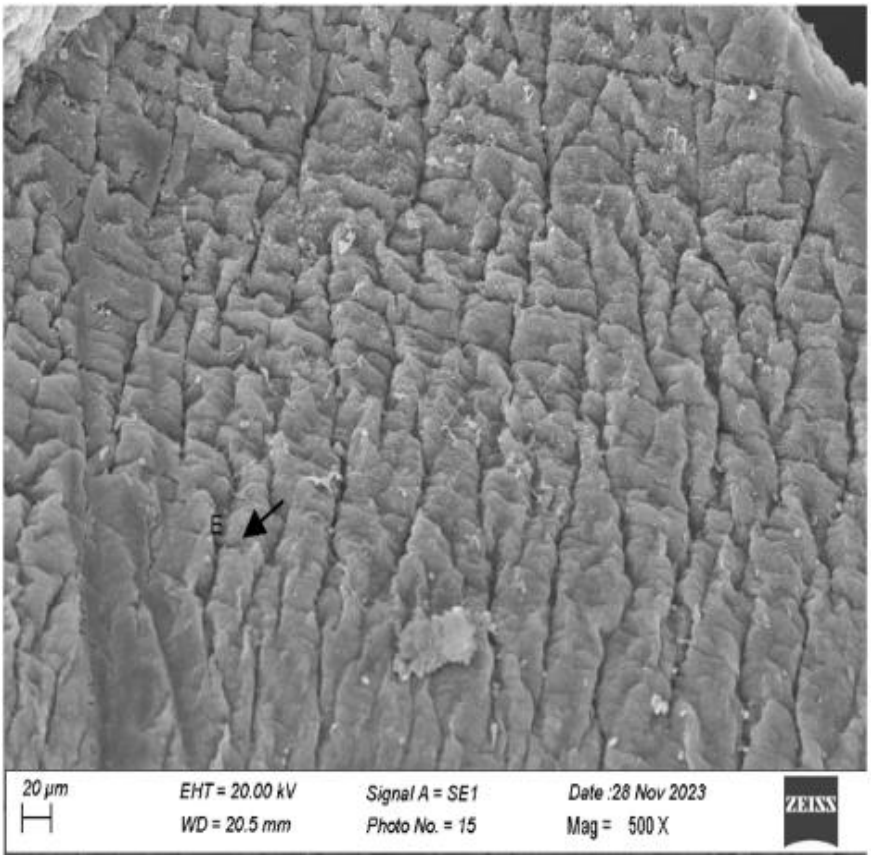

Fig. 1. Micrographic representation of *Heteropneustes fossilis* liver. (Control group), E. Endothelium Magnification 500X

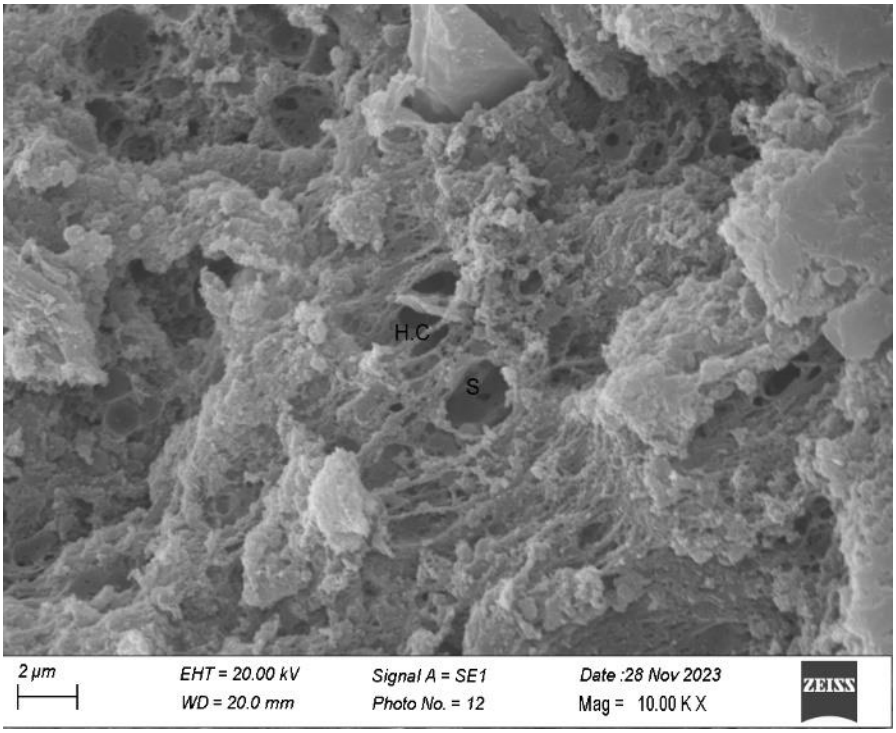

Fig. 2. Micrographic representation of *Heteropneustes fossilis* liver. (Control group), S, Sinusoids, HC Hepatic cord Magnification 10KX

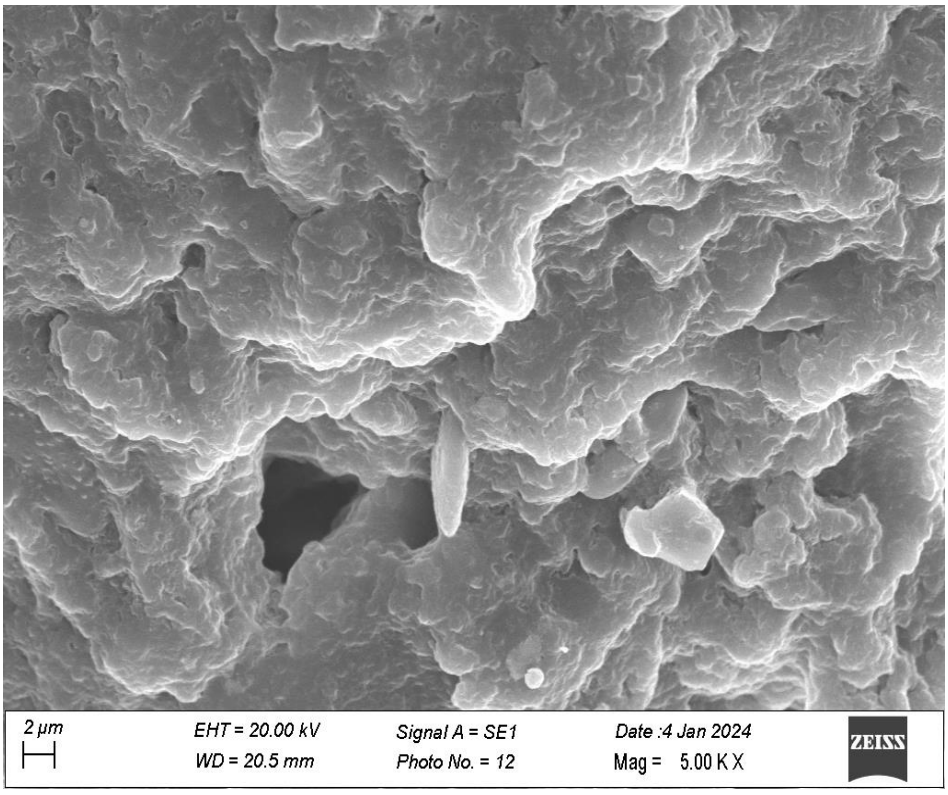

Fig. 3. Micrographic representation of *Heteropneustes fossilis* liver treated with 10µg/L PSNPs. after exposure for 20 days. Magnification 5.00KX

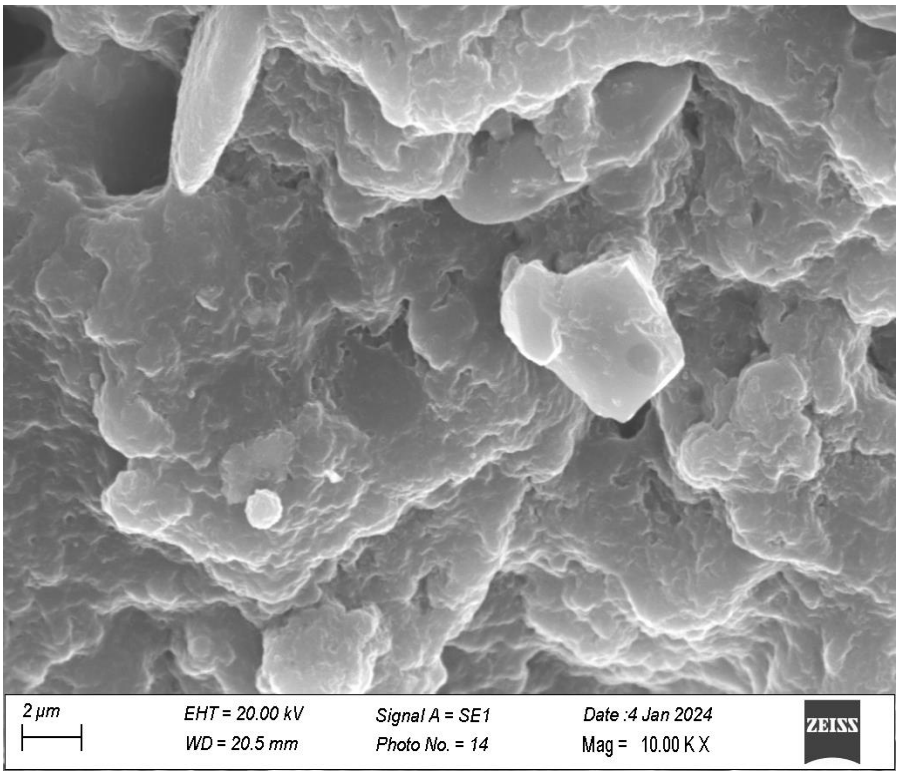

Fig. 4. Micrographic representation of *Heteropneustes fossilis* liver treated with 20µg/L PSNPs. after exposure for 20 days. Magnification 10.00KX

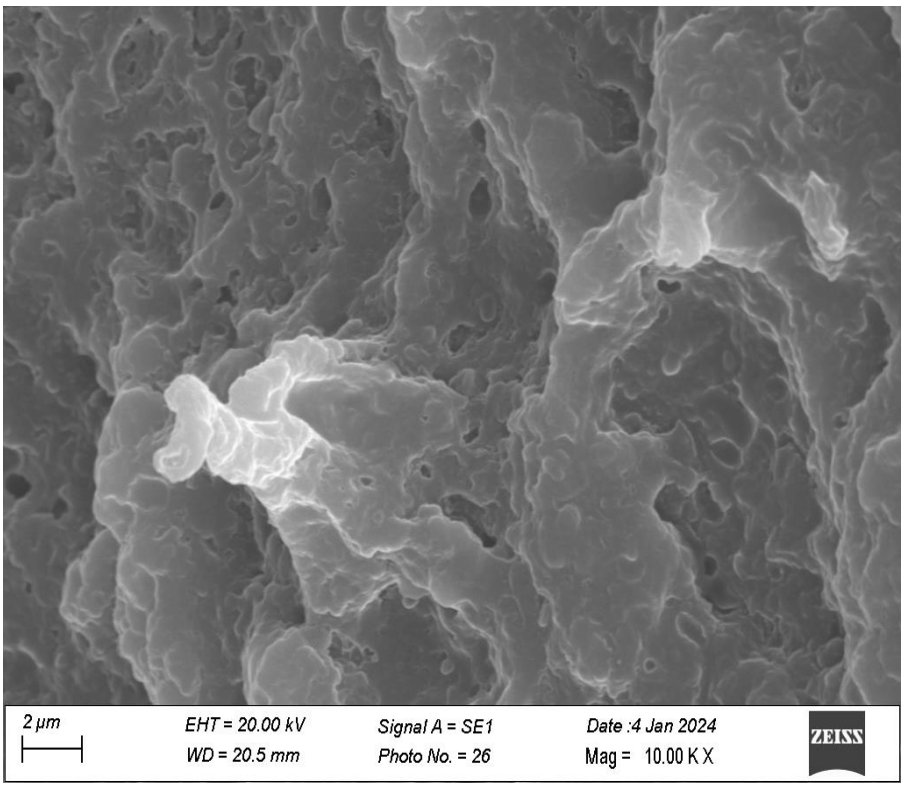

Fig. 5. Micrographic representation of *Heteropneustes fossilis* liver treated with 30µg/L PSNPs. after exposure for 20 days. Magnification 10.00KX

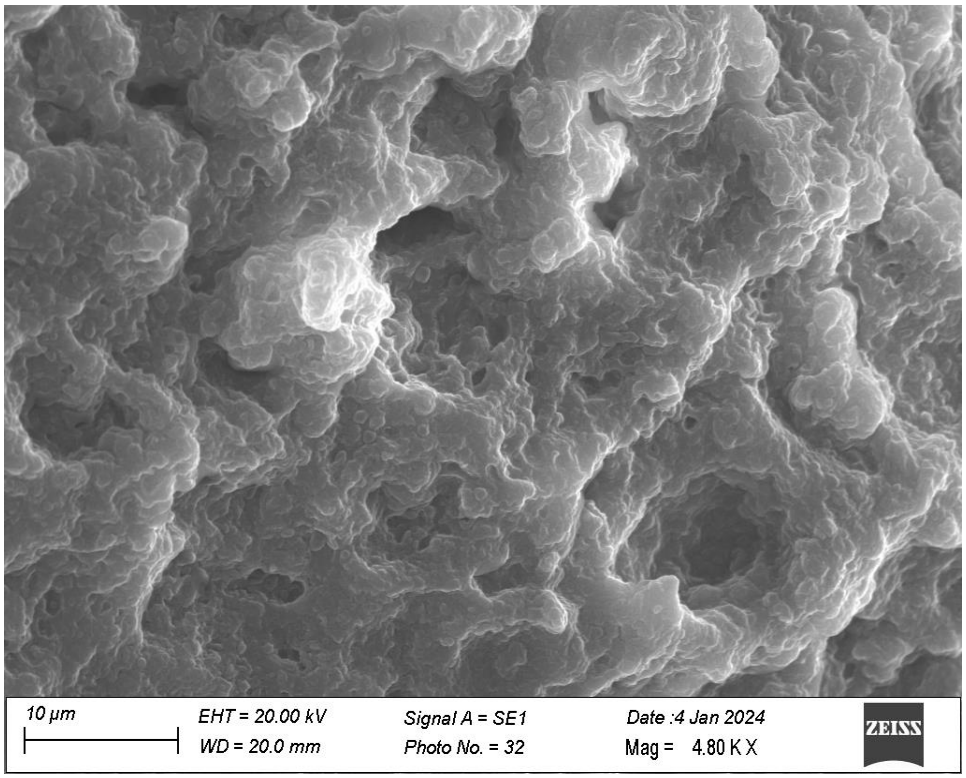

Fig. 6. Micrographic representation of *Heteropneustes fossilis* liver treated with 30µg/L PSNPs. after exposure for 20 days. Magnification 5.00KX

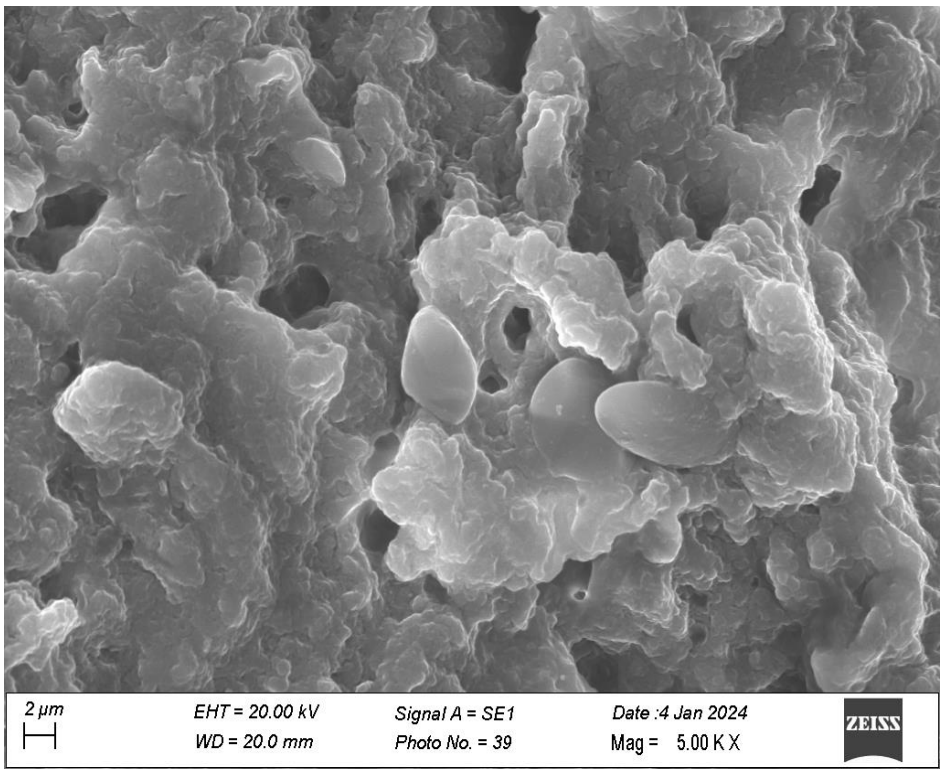

Fig. 7. Micrographic representation of *Heteropneustes fossilis* liver treated with 40µg/L PSNPs. after exposure for 20 days. Magnification 5.00KX

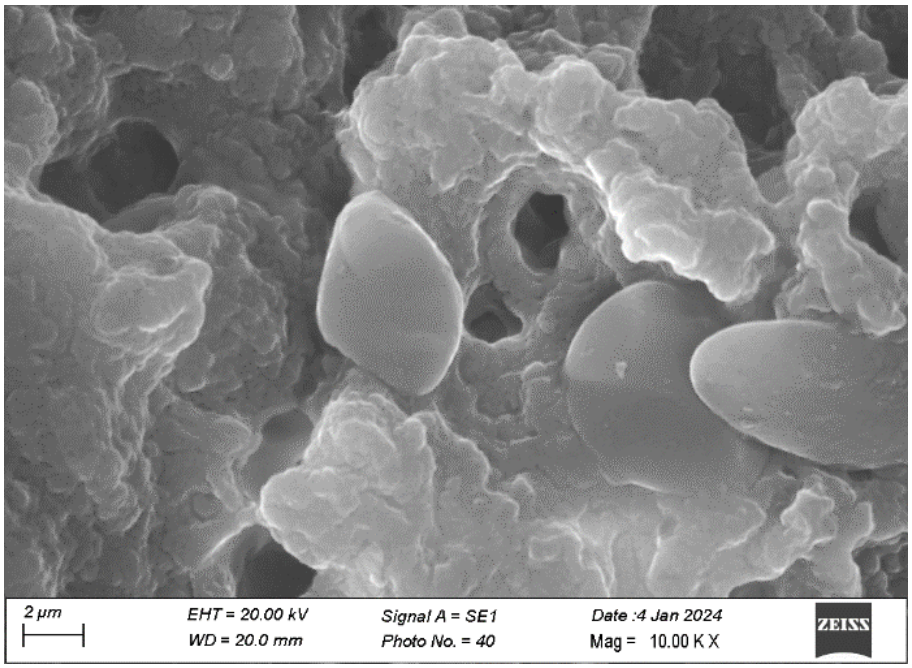

Fig. 8. Micrographic representation of *Heteropneustes fossilis* liver treated with 40µg/L PSNPs. after exposure for 20 days. Magnification 10.00KX

(Fig. 3). Kupffer cells become less in number and cell borders became invisible on exposure of 20µg/L of PS-NPs (Fig. 4). The liver of H. fossilis treated with 30µg/L of PS-NPs, the hepatocytes appeared to float in empty spaces among the hepatocytes (Figs. 5,6). Tremendous number of hepatocytes, blood vessels were ruptured therefore haemorrhage occurred with infiltration of lymphocytes when the liver of *H. fossilis* was treated with 40µg/L of PS-NPs for 20 days. Vacuoles were observed both in extracellular space and in the cytosol (Figs. 7,8).

4. DISCUSSION

Fishes play an important role in aquatic ecosystems. Contamination of fish by plastics especially polystyrene nanoparticles so far studied has brought intense effects on histological structure. In the present study, the effects of Polystyrene Nanoparticles on the liver were examined and progressive histological changes were documented. Several changes in histological structure were noticed under SEM observation during on exposure of PS-NPs in experimental groups. The toxicity of PS-NPs have been affected the liver and showed abnormal structure of liver.

At prolonged exposure of high doses of PS-NPs for 20 days showed varied degrees of hepatic destruction within and around the hepatic parenchyma. Several authors have reported that different toxicants adversely affect the integrity of hepatic cells. Pandey et al. [21], observed that the parenchymal architecture of the liver was disturbed and hepatocytes showed dissociation and granular appearance in the liver of H. fossilis exposed to Pentachlorophenol (PCP). Acute and extensive necrosis of liver cells was observed and the density of connective tissue increased. C.Uguz et al. [22] observed neoplastic proliferation of reticuloendothelial cells in the liver of fish rainbow trout (Onchorynchus mykiss,) exposed to a higher concentration of NP for a long duration (four weeks). The replacement of normal liver tissues by the fibrous septum may be considered as carcinogenic development in the liver [23,24].

Under SEM observation severe damage in hepatocytes and hepatic chords were seen. Alterations in hepatocytes such as vacuolation, necrosis and haemorrhage were observed in the present study. Cytoplasmic vacuolation in hepatocytes indicated decreased protein synthesis due to a lack of lipid-protein conjugation that accompanied hepatic injury [25]. In the liver, severe necrosis in hepatocytes, vacuolization of cytoplasm, degeneration of cell membrane, and severe leukocytes infiltration under SEM study were observed by Samanta et al. [26].

In the present study under SEM observation the liver of the fish exposed to a higher concentration of PS-NPs, hepatic lobules were observed as floated in a empty spaces. The initiation of hepatocellular carcinoma cannot be ruled out with increase in concentration of PS-NPs as the floating hepatocytes marks advent of metastatic state. The accumulation of PS-NPs has caused mechanical damage to the blood vessels and hepatocytes as well leading to infiltration of lymphocytes. The nuclei appear spherical and enlarged [27,28] showing a departure from normal to hepatic malignancy.

5. CONCLUSIONS

The findings of this study provide strong evidence that exposure to polystyrene nanoparticles can severely damage the liver of H. fossilis over an extended period. The histological findings demonstrate that the health status of fish can serve as a reliable indicator for assessing the level of contamination in aquatic environments. Therefore, it is imperative to closely monitor and regulate the release of PS-NPs to safeguard the health of aquatic organisms and preserve the integrity of aquatic ecosystems. Besides the plastic pollution calls for special attention as the fragmented particles can reach to human beings in subtle manner through several ways to cause acute histopathological conditions.

ETHICAL APPROVAL

The protocol was approved by the Ethical Committee of University Department of Zoology, SKMU, Dumka.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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